22 pts received up to 11 cycles of extended treatment. The safety profile of rIL-21 was similar in the 2 studies and 2 patient populations. rIL-21 was well tolerated at doses of 1–10 μg/kg. Overall, the most commonly reported adverse events were fatigue, pyrexia, chills, nausea, and rash. In the 3/w regimen 30 μg/kg was declared the MTD based on DLTs in 3/7 pts and no higher doses were studied. The MTD for the 5+9 regimen was estimated to be 30 μg/kg in both studies though higher doses of 50 and 100 μg/kg were tolerated by some pts. Immunomodulatory effects were observed at all dose levels with increased levels of phosphorylated STAT3 even at the 1 μg/kg dose level; increased soluble CD25; increases in NK, CD8+ and CD4+ cells; and upregulation of perforin and granzyme-A & -B mRNA at doses ⩾3 μg/kg. Three pts (all RCC) achieved confirmed PRs and two pts (both MM, one previously treated in a vaccine study) achieved CRs according to RECIST after up to 11 cycles of treatment.
Conclusions: Based on data from 72 pts with MM or RCC exposed to rIL-21 in two phase 1 studies, rIL-21 was generally well tolerated. Relevant biological activity was observed at doses as low as 1 μg/kg. Four responses of 50 evaluable pts treated at doses ⩾30 μg/kg provide encouragement for future studies of rIL-21.

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Preclinical evaluation of IL-21 combination therapy with sorafenib and sunitinib in renal cell carcinoma

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Background: Sorafenib and sunitinib are tyrosine kinase inhibtors (TKIs) recently approved for the treatment of advanced RCC. Mechanisms of TKI-mediated tumor inhibition include direct inhibition of tumor cell proliferation and inhibition of angiogenesis via VEGF and PDGF pathways. While both drugs have demonstrated meaningful clinical benefit in RCC, most patients will relapse. The efficacy of TKIs may be improved in combination with other agents. IL-21 is a novel cytokine that has shown potent efficacy in preclinical models of RCC through mechanisms involving activation of NK cells and tumor-specific CD8 T cells. A Phase 1 study showed IL-21 to be tolerated as an outpatient regimen in RCC and pharmacologically active. Combining IL-21 with TKIs may result in greater clinical benefit by influencing multiple independent pathways. A series of preclinical pharmacology studies were designed with the objective of characterizing the potential pharmacologic interactions of TKIs and IL-21.
Methods: The effects of sorafenib and sunitinib on IL-21-mediated effector functions were tested under conditions of concurrent or sequential exposure using a range of concentrations of TKIs including steady state and maximal levels reported in patients. Assays employed measured NK cell cytotoxicity and IFNγ production, and IL-21 co-stimulation of CTL proliferation. Furthermore, effects of TKIs on IL-21R expression and STAT3 phosphorylation on PBMCs were evaluated. In vivo studies with murine IL-21 and TKIs were performed in subcutaneous B16 melanoma and RenCa RCC in mice.
Results: At steady-state concentrations of drug reported in serum of human patients, neither sorafenib nor sunitinib inhibited IL-21R expression or IL-21-induced STAT3 phosphorylation in human PBMCs, human or mouse CD4 and CD8 T cell proliferation, human NK cell granzyme B expression and ADCC activity. IL-21 treatment did not affect sunitinib or sorafenib-mediated anti-tumor effects in the syngeneic tumor models at maximal doses of TKIs. Additive anti-tumor effects were observed with IL-21 in combination with sub-maximal sorafenib.
Conclusions: Preclinical evaluation of the combination of TKIs and IL-21 suggests that the TKIs, when used at concentrations simulating therapeutic exposure, do not inhibit IL-21 or immune effector functions in vitro. Further, IL-21, in combination with TKI has additive effects in preclinical models, suggesting that testing of IL-21 and TKIs clinically is warranted.

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A chimera of interleukin-2 and a variant of the channel-forming protein aerolysin is selectively toxic to cells displaying the interleukin-2 receptor

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Background: Proaerolysin is the inactive precursor of the bacterial toxin aerolysin. The protoxin binds to GPI-anchored proteins (GPI-AP) on mammalian cells and is converted to aerolysin by proteolytic nicking. Aerolysin then spontaneously forms a stable oligomer that inserts into the plasma membrane and forms channels that cause cell death. Once inserted into the membrane, the toxin cannot leave, so that bystander cells cannot be affected: thus aerolysin may provide an advantage over enzyme toxins such as diphtheria toxin and exotoxin A, as a component of hybrid molecules that can target specific cell types.
Methods: To prevent binding of proaerolysin to normal cells, we made the variant R336A-PA. The R336 residue was identified based on our previous studies of proaerolysin binding to GPI-anchored proteins and the known structure of the protoxin. We also made a hybrid (IL-2-PA) of IL-2 fused to native proaerolysin and a second hybrid (IL-2-R336A-PA), by fusing IL-2 to R336A-PA. A six amino acid spacer separated the IL-2 and the proaerolysin. We determined whether the proaerolysin forms of these molecules could be converted to the aerolysin forms by proteolytic nicking, as well as the ability of the aerolysin forms to produce stable oligomers. Flow cytometry was used to compare binding of R336A-PA and the two hybrid molecules to cells displaying the IL-2 receptor and to cells that do not. Cell killing was studied using a variety of cell lines.
Results: We showed that all of the molecules could be converted to the aerolysin form by proteolytic nicking and that this led to the production of stable oligomers. The R336A variant of proaerolysin did not bind and was only very weakly active against all cell types tested. The IL-2-PA hybrid was active against all cell types, as it could bind to GPI-AP and form functional oligomers. The Il-2-R336A-PA hybrid could not bind to normal GPI-AP positive cells and it had little or no activity against them. Remarkably, this hybrid could bind to cells that display the IL-2 receptor and it was nearly as toxic to these cells as native PA.
Conclusions: The channel-forming protein aerolysin can be targeted to cells displaying the IL-2 receptor. Targeted aerolysin molecules such as IL-2-R336A-PA may have advantages over targeted enzyme toxin molecules in cancer therapy.

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Cancer immunotherapy by Interleukin-21: theoretical evaluation of potential treatment strategies

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Background: The newly-characterized Interleukin-21 (IL-21), a natural derivative of T-helper cells, plays a central role in the transition from innate immunity to adaptive immunity. Murine studies show substantial elimination of various tumors in response to IL-21 application, thereby encouraging its addition into the growing cancer immunotherapeutic arsenal. Still, conditions for efficacious IL-21-therapy, and its conflicting immunostimulatory and immunoinhibitory influence on anticancer cellular responses, are yet to be fully defined.
Methods: We have studied the effects of IL-21 on tumor eradication in a mathematical model focusing on the NK-cell and CD8+ T cell-mediated lysis of tumor cells. To estimate model parameters we used studies in mice inoculated with poorly immunogenic (PI) melanomas, highly immunogenic (HI) fibrosarcomas, or thymoma, and treated with cytokine gene therapy (CGT), hydrodynamics-based gene delivery (HGD), or standard interval dosing (SID) of IL-21. Model accuracy in retrieving tumor growth curves has been validated in independent experiments of melanoma and fibrosarcoma progression in mice treated by IL-21. Putative immunotherapy strategies were simulated and their efficacy was estimated.
Results: Computer simulations accurately retrieved experimental growth dynamics in B16 melanoma, MethA and MCA205 fibrosarcomas, showing a strong dependence of the NK-cell/CD8+ T-cell balance on tumor immunogenicity. Efficient tumor elimination was achieved in melanoma, when simulating an IL-21 dosing regimen that was dynamically-determined according to changes in tumor mass, as in CGT. In contrast, in fibrosarcoma, such a strategy did not prove superior to that of constant dosing protocols, HGD or SID.
Conclusions: Our model analysis supports clinical use of IL-21 as a potent stimulator of cellular immunity against cancer, and suggests selecting the immunotherapy strategy according to tumor immunogenicity. In PI malignancies, but not in HI, IL-21 dosing, at any time, should depend on tumor mass at that time. This method imitates, yet amplifies, the natural anticancer immune response, rather than accelerating only one of the response arms, in an unbalanced manner.

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Targeting brain tumor stem cells with oncolytic virus in combination with temozolomide

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Background: Recently, several groups have described the existence of a cancer stem cell population in human brain tumors. These population is a preferred therapeutic target since has been proposed to be a possible